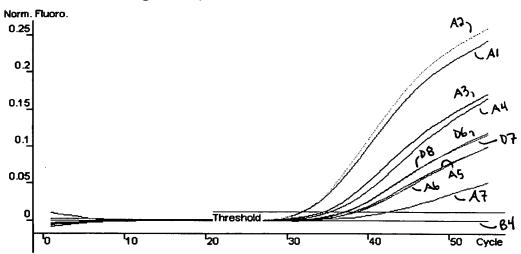
Title: Method And Kit For A Nuclear Run-On Assay (AS AMENDED)

Inventor: Robert N. RICE et al. Application No.: 10/081,646 Docket No.: 546322000100

Amplification pl ts and Quantitation data for Human BRN2 (Duplexed with Human GAPDH Figur 1b)



Standard Curve



10^-	1.	10^0		10^2		Concentration		
No.	Colou	Name	Туре	Given Conc.	Calculated Conc.	CV	Ct	Ct Std. Dev.
A1		dT SS RNA 200ng	Standard	200.0	174.9	12.54%	31.94	0.07
A2		dT SS RNA 200ng	Standard	200.0	187.5	6.25%	31.79	0.07
A3		dT SS RNA 40ng	Standard	40.0	54.2	35.54%	34.47	0.43
A4		dT SS RNA 40ng	Standard	40.0	36.4	8.98%	35.33	0.43
A5		dT SS RNA 8ng	Standard	8.0	7.9	1.70%	38.64	0.13
A6		dT SS RNA 8ng	Standard	8.0	8.9	10.88%	38.38	0.13
A7		dT SS RNA 1.6ng	Standard	1.6	1.5	9.30%	42.29	21.15
A8		dT SS RNA 1.6ng	Standard	1.6	***			21.15
B4		RTminus MM96L 2.1.1, NRO 10 ⁶ nuclei	Sample					
D6		RT plus MM96L 2.1.1 , NRO 10 ⁶ nuclei	Sample		16.3		37.07	
D7		RT plus MM96L 2.1.1 , NRO 10 ⁶ nuclei	Sample		14.5		37.32	
D8		RT plus MM96L 2.1.1 , NRO 10 ⁶ nuclei	Sample		10.1		38.11	

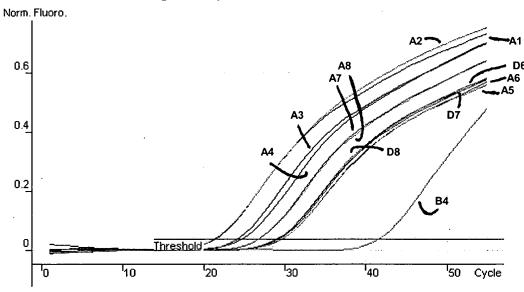
Figure 1a

Title: Method And Kit For A Nuclear Run-On Assay (AS AMENDED)

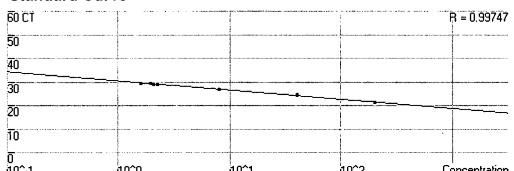
Inventor: Robert N. RICE et al. Application No.: 10/081,646 Docket No.: 546322000100







Standard Curve

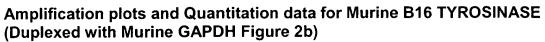


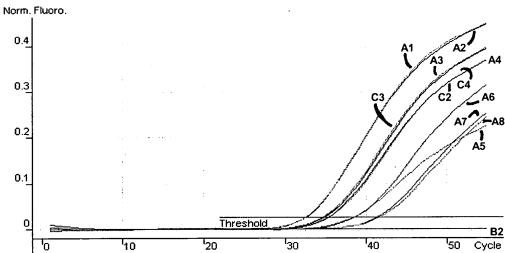
10^-1	10^0		(10^1	<u>0^1 i10^2</u>		Concentration		
	Colo	ur Name	Type	Given Conc.	Calculated Conc.	cv	Ct	Ct Std. Dev.
A1		dT SS RNA 200ng	Standard	200.0	210.2	5.10%	21.37	0.04
A2	F.	dT SS RNA 200ng	Standard	200.0	220.4	10.18%	21.29	0.04
A3		dT SS RNA 40ng	Standard	40.0	40.3	0.78%	24.17	0.29
A4		dT SS RNA 40ng	Standard	40.0	28.8	28.00%	24.74	0.29
A5		dT SS RNA 8ng	Standard	8.0	8.4	5.57%	26.82	0.01
A6		dT SS RNA 8ng	Standard	8.0	8.5	6.82%	26.8	0.01
A7		dT SS RNA 1.6ng	Standard	1.6	1.6	0.55%	29.65	0.06
A8		dT SS RNA 1.6ng	Standard	1.6	1.7	6.12%	29.54	0.06
B4		RTminus MM96L 2.1.1 NRO 10 ⁶ nuclei	Sample		0.0		41.63	
D6		RT plus MM96L 2.1.1 NRO 10 ⁶ nuclei	Sample		2.3		29.05	
D7		RT plus MM96L 2.1.1 NRO 10 ⁶ nuclei	Sample		2.0		29.29	
D8		RT plus MM96L 2.1.1 NRO 10 ⁶ nuclei	Sample		2.1		29.18	

Figure 1b

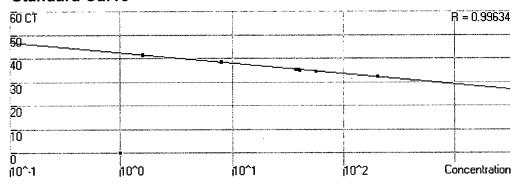
Title: Method And Kit For A Nuclear Run-On Assay (AS AMENDED)

Inventor: Robert N. RICE et al. Application No.: 10/081,646 Docket No.: 546322000100





Standard Curve



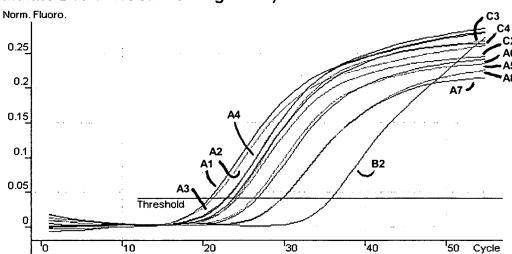
No.	Colour	Name	Туре	Given Conc.	Calculated Conc.	cv	Ct	Ct Std. Dev.
A1		dT SS RNA 200ng	Standard	200.0	166.8	16.60%	32.52	0.05
A2		dT SS RNA 200ng	Standard	200.0	175.7	12.16%	32.42	0.05
A3		dT SS RNA 40ng	Standard	40.0	52.2	30.55%	34.76	0.06
A4		dT SS RNA 40ng	Standard	40.0	49.1	22.67%	34.88	0.06
A5		dT SS RNA 8ng	Standard	8.0	7.9	1.12%	38.4	0.10
A6		dT SS RNA 8ng	Standard	8.0	7.1	10.86%	38.6	0.10
A7		dT SS RNA 1.6ng	Standard	1.6	1.7	6.56%	41.36	0.16
A8		dT SS RNA 1.6ng	Standard	1.6	1.5	9.26%	41.67	0.16
B2		RTminus B16 TYR Parental NRO 10 ⁶ nuclei	Sample					
C2		RT plus B16 TYR parental NRO 10 ⁶ nuclei	Sample		37.1		35.42	
C3		RT plus B16 TYR parental NRO 10 ⁶ nuclei	Sample		56.2		34.62	
C4		RT plus B16 TYR parental NRO 10 ⁶ nuclei	Sample		39.1		35.32	

Figure 2a

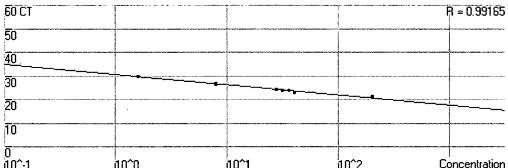
Title: Method And Kit For A Nuclear Run-On Assay (AS AMENDED)

Inventor: Robert N. RICE et al. Application No.: 10/081,646 Docket No.: 546322000100

Amplification plots and Quantitation data for GAPDH (Duplexed with Murine B16 TYROSINASE Figure 2a)



Standard Curve



110 -	l	ון ט טון	<u>U I </u>		Concentration			
No.	Colour	Name	Type	Given Conc.	Calculated Conc.	CV	Ct	Ct Std. Dev.
A1		dT SS RNA 200ng	Standard	200.0	168.4	15.81%	20.91	0.20
A2		dT SS RNA 200ng	Standard	200.0	136.9	31.55%	21.3	0.20
A3		dT SS RNA 40ng	Standard	40.0	53.5	33.75%	23.07	0.07
A4		dT SS RNA 40ng	Standard	40.0	57.6	44.07%	22.93	0.07
A5		dT SS RNA 8ng	Standard	8.0	9.2	14.78%	26.39	0.19
A6		dT SS RNA 8ng	Standard	8.0	7.5	6.19%	26.77	0.19
A7		dT SS RNA 1.6ng	Standard	1.6	1.5	7.08%	29.82	0.03
A8		dT SS RNA 1.6ng	Standard	1.6	1.4	9.99%	29.88	0.03
B2		RTminus B16 TYR Parental NRO 10 ⁶ nuclei	Sample		0.1		35.94	
C2		RT plus B16 TYR parental NRO 10 ⁶ nuclei	Sample		27.8		24.3	
C3		RT plus B16 TYR parental NRO 10 ⁶ nuclei	Sample		31.1		24.09	
C4		RT plus B16 TYR parental NRO 10 ⁶ nuclei	Sample		35.9		23.82	

Figure 2b

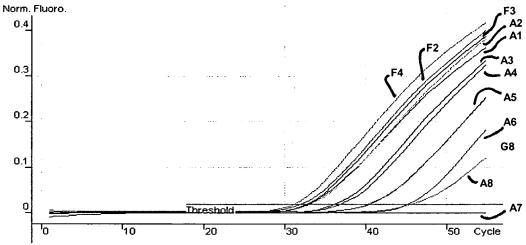


Title: Method And Kit For A Nuclear Run-On Assay (AS AMENDED)

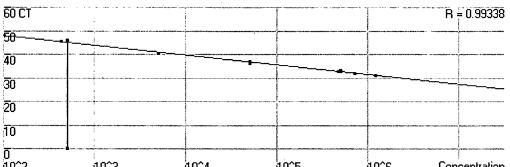
Inventor: Robert N. RICE et al. Application No.: 10/081,646 Docket No.: 546322000100

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Amplification plots and Quantitation data for EGFP (Duplexed with Murine GAPDH Figure 3b)



Standard Curve

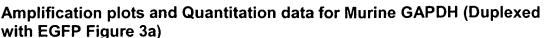


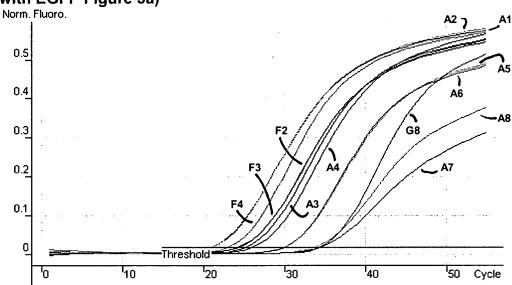
<u> 1022</u>			<u>,1013 1014 </u>		<u>10^5 10^6 </u>		Loncentration		
No.	Cold	our	Name	Туре	Given Conc.	Calculated Conc.	cv	Ct	Ct Std. Dev.
A1			dT/SS RNA 500ng	Standard	500,000	438,882	12.22%	32.97	0.12
A2	Wie '		dT/SS RNA 500ng	Standard	500,000	381,732	23.65%	33.22	0.12
A3			dT/SS RNA 50ng	Standard	50,000	65,817	31.63%	36.37	0.33
A4			dT/SS RNA 50ng	Standard	50,000	45,539	8.92%	37.03	0.33
A5			dT/SS RNA 5ng	Standard	5,000	7,062	41.23%	40.37	0.07
A6			dT/SS RNA 5ng	Standard	5,000	6,531	30.62%	40.51	0.07
A7			dT/SS RNA 0.5ng	Standard	500				22.91
A8			dT/SS RNA 0.5ng	Standard	500	337	32.53%	45.82	22.91
F2			RT plus B16 EGFP #12 NRO 10 ⁶ nuclei	Sample		717,169		32.09	
F3			RT plus B16 EGFP #12 NRO 10 ⁶ nuclei	Sample		477,201		32.82	
F4			RT plus B16 EGFP #12 NRO 10 ⁶ nuclei	Sample		1,198,365		31.17	
G8			RT minus B16 EGFP #12 NRO 10 ⁶ nuclei	Sample		429		45.39	

Figure 3a

Title: Method And Kit For A Nuclear Run-On Assay

(AS AMENDED) Inventor: Robert N. RICE et al. Application No.: 10/081,646 Docket No.: 546322000100







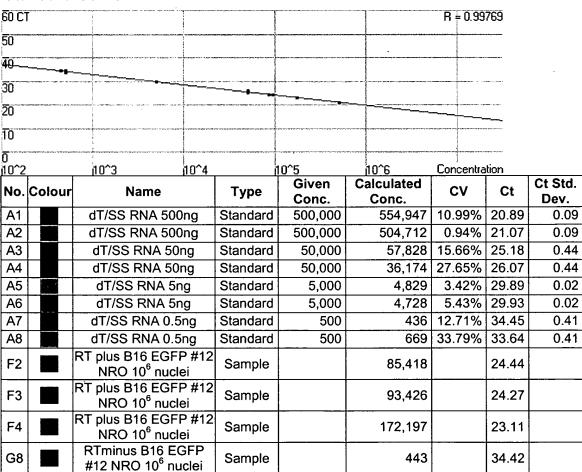


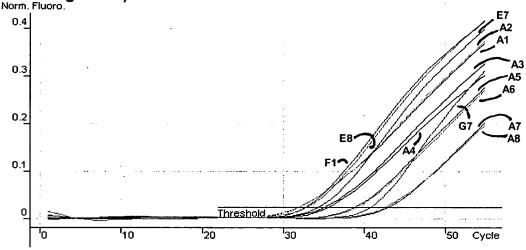
Figure 3b

Title: Method And Kit For A Nuclear Run-On Assay (AS AMENDED)

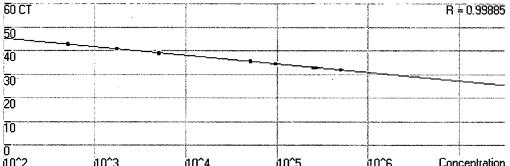
Inventor: Robert N. RICE et al. Application No.: 10/081,646 Docket No.: 546322000100



Amplification plots and Quantitation data for EGFP (Duplexed with Human GAPDH Figure 4b) Norm. Fluoro.



Standard Curve



10^2	2			10.3	₁ 10^4	(10^5)	i10^6	Concentration	on	
No.	С	olo	ur	Name		Type	Given Conc.	Calculated Conc.	CV	Ct	Ct Std. Dev.
A1				dT/SS RNA 5	00ng	Standard	500,000	475,544	4.89%	31.94	0.01
A2				dT/SS RNA 5	00ng	Standard	500,000	481,621	3.68%	31.92	0.01
A3				dT/SS RNA	50ng	Standard	50,000	60,025	20.05%	35.2	0.26
A4				dT/SS RNA	50ng	Standard	50,000	43,148	13.70%	35.72	0.26
A5		€		dT/SS RNA	5ng	Standard	5,000	4,889	2.22%	39.15	0.15
A6				dT/SS RNA	5ng	Standard	5,000	5,877	17.55%	38.86	0.15
A7	Ī			dT/SS RNA ().5ng	Standard	500	422	15.67%	43.01	0.20
A8	Г			dT/SS RNA 0).5ng	Standard	500	544	8.71%	42.61	0.20
E7				RT Plus MM96L #22 NRO 10 ⁶		Sample		94,810		34.48	
E8				RT Plus MM96L #22 NRO 10 ⁶		Sample		244,164		32.99	
F1				RT Plus MM96L #22 NRO 10 ⁶		Sample		265,171		32.86	
G7				RT minus MM96 #22 NRO 10 ⁶		Sample		1,759		40.76	

Figure 4a

Title: Method And Kit For A Nuclear Run-On Assay

(AS AMENDED) Inventor: Robert N. RICE et al. Application No.: 10/081,646 Docket No.: 546322000100



0.06

0.06

0.21

0.21

0.28

0.28

0.16

0.16

24.62

22.56

22.82

38.17

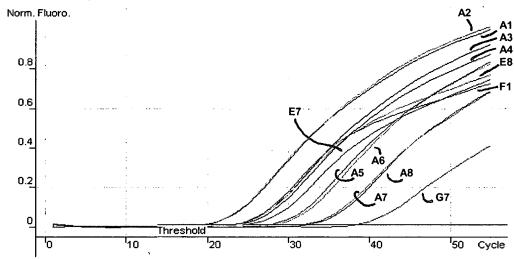
26,434

81,772

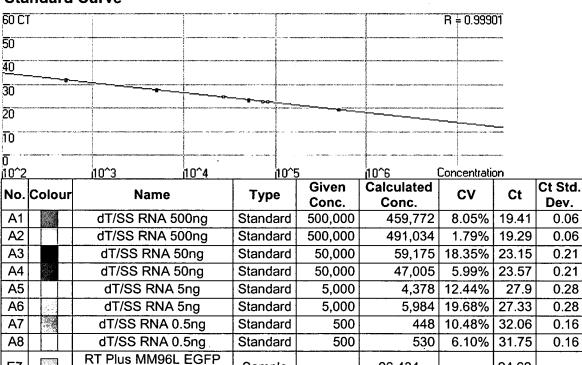
70,909

16

Amplification plots and Quantitation data for Human GAPDH (Duplexed with EGFP Figure 4a)



Standard Curve



Sample

Sample

Sample

Sample

#22 NRO 10⁶ nuclei RT Plus MM96L EGFP

#22 NRO 10⁶ nuclei RT Plus MM96L EGFP

#22 NRO 10⁶ nuclei RT minus MM96L EGFP

#22 NRO 10⁶ nuclei

Figure 4b

E7

E8

F1

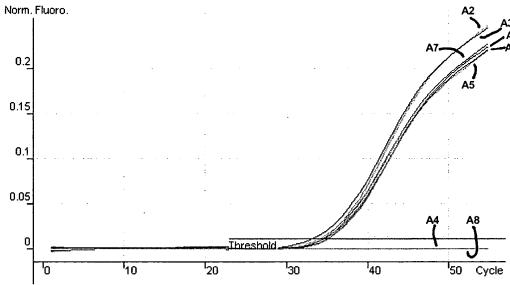
G7

Title: Method And Kit For A Nuclear Run-On Assay (AS AMENDED)
Inventor: Robert N. RICE et al.

Inventor: Robert N. RICE et al. Application No.: 10/081,646 Docket No.: 546322000100



Amplification plots and Quantitation data for Human Endogenous HER2 (Duplexed with Human GAPDH Figure 5b)

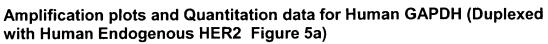


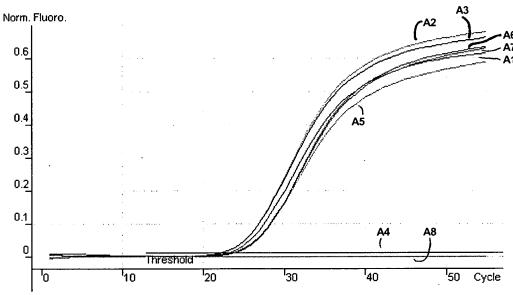
No.	Colour	Name	Туре	Ct	Ct Std. Dev.
A1		NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #2.6	Sample	34.63	
A2		NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #2.6	Sample	34.47	0.67
А3		NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #2.6	Sample	33.4	
A4		NRO 10 ⁶ nuclei RTminus MDA-MB 468 HER2 positive clone #2.6	Sample		
A5	10	NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #4.3	Sample	34.22	
A6		NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #4.3	Sample	34.74	0.47
A7		NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #4.3	Sample	35.16	
A8		NRO 10 ⁶ nuclei RTminus MDA-MB 468 HER2 positive clone #4.3	Sample		

Figure 5a

Title: Method And Kit For A Nuclear Run-On Assay

(AS AMENDED)
Inventor: Robert N. RICE et al.
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Docket No.: 546322000100





No.	Colour	Name	Туре	Ct	Ct Std. Dev.
A1		NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #2.6	Sample	23.16	
A2		NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #2.6	Sample	22.35	0.48
А3		NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #2.6	Sample	22.31	
A4		NRO 10 ⁶ nuclei RTminus MDA-MB 468 HER2 positive clone #2.6	Sample		
A5		NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #4.3	Sample	23.77	
A6		NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #4.3	Sample	23.71	0.18
A7		NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #4.3	Sample	24.05	
A8		NRO 10 ⁶ nuclei RTminus MDA-MB 468 HER2 positive clone #4.3	Sample		

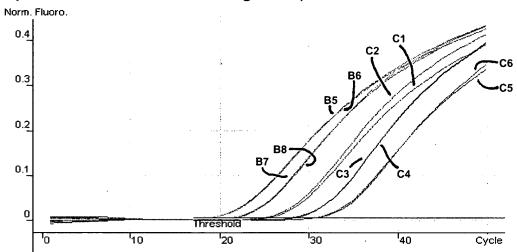
Figure 5b

Title: Method And Kit For A Nuclear Run-On Assay

(AS AMENDED) Inventor: Robert N. RICE et al. Application No.: 10/081,646 Docket No.: 546322000100



Amplification plots and Quantitation data for HER-2 Exogenous assay (Duplexed with Human GAPDH Figure 6b)



Standard Curve

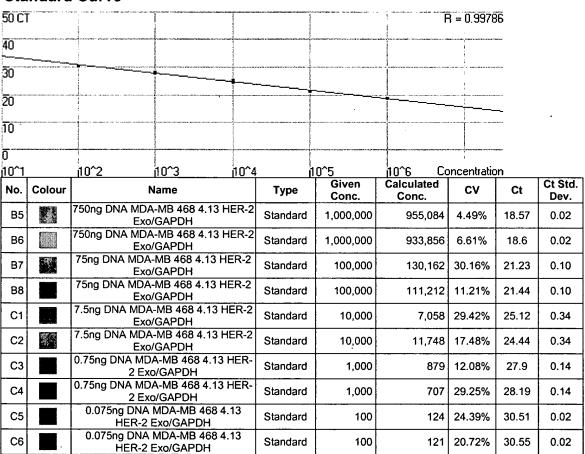


Figure 6a

Title: Method And Kit For A Nuclear Run-On Assay

(AS AMENDED)

Inventor: Robert N. RICE et al. Application No.: 10/081,646 Docket No.: 546322000100



Amplification plots and Quantitation data for Human GAPDH (Duplexed with HER-2 Exogenous assay Figure 6a)

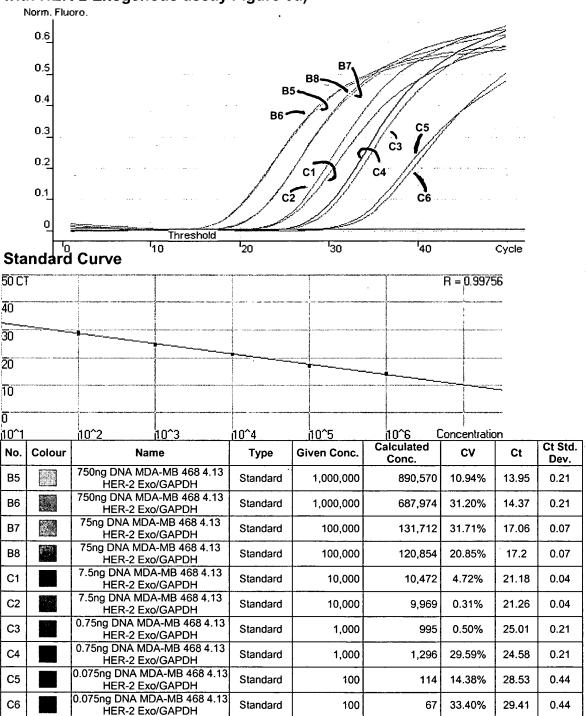


Figure 6b